The Open Ophthalmology Journal

Content list available at: https://openophthalmologyjournal.com

RESEARCH ARTICLE

The Thiol-Disulfide Homeostasis and Coenzyme Q10 in Conjunction with Vitamin E Effect on Retinopathy Prematurity

Muberra Akdogan[1,*], Yasemin Ustundag[2], Arzu Akdağ[3], Salim Neselioglu[4] and Ozcan Erel[4]

[1]*Department of Ophthalmology, Faculty of Medicine, Afyonkarahisar Health Science University, Afyonkarahisar 03320, Turkey*
[2]*Department of Clinical Biochemistry, University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa 16290, Turkey*
[3]*Newborn Clinic, University of Health Sciences, Bursa Yuksek Ihtisas Training and Research Hospital, Bursa 16290, Turkey*
[4]*Department of Clinical Biochemistry, Yildirim Beyazit University, 06760, Ankara, Turkey*

Abstract:

Purpose:

This study was performed to determine whether one drop of topical administration of Coqun® (Coenzyme Q10 and Vitamin E)-a potent antioxidant-twice a day has any effect on the thiol-disulphide homeostasis-a novel oxidative stress marker in the Retinopathy Of Prematurity (ROP) disease course.

Methods:

This was a prospective observational study comprising 28 infants with ROP at stage 2 and higher who followed up at the paediatric intensive care unit. Ferric reducing power of plasma (FRAP), albumin, ischemia-modified albumin (IMA) and thiol disulphide homeostasis levels were studied in the infants before and two weeks after Coqun® treatment.

Results:

The mean gestational age was 27 (24–32) weeks, the mean birth weight was 1,012±326 g and the mean duration of care in an incubator was 64±23 days. FRAP levels were 0.91±0.17 µmol/L, IMAs were 0.85±0.29, native thiols were 248±38.9 µmol/L and total thiols were 284±39.2 µmol/L, respectively, at the beginning of therapy.

FRAP levels 0.79±0.21(p= 0.006) µmol/L, IMAs 0.73±0.36(p = 0.096), native thiols 262±42.6(p = 0.164) µmol/L and total thiols 291±43.6(p = 0.344) µmol/L showed no difference after two weeks of therapy.

Conclusion:

Thiol disulphide homeostasis levels do not change with Coqun® therapy during ROP course.

Keywords: Antioxidants, Coenzyme Q10, Retinopathy of Prematurity, Thiol, Vitamin E, FRAP.

Article History	Received: December 13, 2018	Revised: February 07, 2019	Accepted: March 06, 2019

1. INTRODUCTION

Retinopathy Of Prematurity (ROP) is a proliferative vasculopathy that usually develops in infants with low birth weight and low gestational age because of vascular dysfunction of the retina. Numerous factors have been proposed as aetiology of this disease, which causes structural impairment in immature retinal vessels and may result in blindness. The most significant factors are oxidative stress caused by oxygen (O_2) and O_2 metabolites [1, 2] and associated inflammation [3]. Studies demonstrating that Hb and Hb products, as well as haemogram parameters, are responsible for O_2 transport can be effective on ROP in infants, as they support the role of oxidative stress in ROP development [4].

Even full-term newborns may be exposed to oxidative stress as a result of exposure to high O_2 content in the natural environment compared with that in the mother's womb. Oxidative stress represents the imbalance between the production of Reactive Oxygen Species (ROS) and protective antioxidants [5]. Oxidative stress and oxidative damage due to

* Address correspondence to this author at the Department of Ophthalmology, Faculty of Medicine, Afyonkarahisar Health Science University, Afyonkarahisar 03320, Turkey; E-mail: mbrakdogan@yahoo.com

insufficient antioxidative response mechanisms in preterm infants are much higher than those in normal infants. In addition, because oxygen in the retina is supplied from the choroid, hyperoxygenation is observed in the inner and outer retina due to the lack of autoregulation between the choroid and the retina as well as inadequate antioxidant defence mechanism, particularly in premature infants. Hyperoxygenation causes oxidative stress in the retina, which plays an important role in ROP development [6 - 8]. Expression of Vascular Endothelial Growth Factor (VEGF), which causes neovascularisation in the retina, is regulated by signals associated with hypoxia [9]. Oxidative stress also plays a role in pathological processes, including the regulation of VEGF expression in the retina [10].

Neonatal Intermittent Hypoxia (IH) is a recurrent, short-term (<3 min) decrease in oxygen saturation [11]. IH episodes are typically a consequence of an immature respiratory control system, and may result in the disruption of the antioxidant system, particularly in premature infants, leading to increased retinal neovascularisation. IH-induced mitochondrial ROS are toxic, causing lipid peroxidation and damaging cell membranes [11].

Coenzyme Q10 (CoQ10) is a potent antioxidant that plays a vital role in energy creation, electron transport and the mitochondrial electron transport chain. CoQ10 dissolves in fat and is often found in high-energy consuming tissues, such as the brain, eyes, liver and kidneys. The antioxidant is a naturally-occurring compound, primarily playing a bioenergenetic role in the cell [12]. CoQ10 has been suggested to inhibit the developmental deficits caused by IH-induced oxidative stress [11].

Thiols are functional sulfhydryl groups that are contained within proteins and other molecules, such as glutathione and homocysteine. Thiol groups are converted to disulphide bonds under oxidative conditions, such as IH and the resulting bonds are degraded to thiols to create thiol-disulphide homeostasis. Thiol-disulphide homeostasis plays an important role in the maintenance of many physiological processes necessary for organisms, such as antioxidant defence and protein chemical structure stabilisation. A method developed in recent years makes it possible to measure thiol-disulphide homeostasis in the blood [13, 14].

Recently. Unal *et al*. suggested that the shift in the thiol-disulfide equilibrium towards disulphides in the first week can be attributed to the subjection of very low-birth-weighted preterms to oxidative stress [15].

This study was performed to determine whether one drop of topical administration of Coqun®, a potent antioxidant consisting of CoQ10 0.1% (w/v) and vitamin E TPGS (d-alpha-tocopheryl polyethylene glycol 1000 succinate) 0.5% (w/v) dissolved in aqueous saline isotonic solution [16], twice a day has any effect on the thiol-disulphide homeostasis in the ROP disease course.

2. METHODS

Twenty-eight infants with a mean birth weight of 1,021 ± 330 g and a mean gestational age of 27.5 ± 2.0 weeks who followed up in the intensive care unit of our hospital between August 2018 and September 2018 for ROP were included in this study. This was a prospective observational case-control study; approval was obtained from the Ethics Committee of HSU Bursa Yuksek Ihtisas Training and Research Hospital. Infants were included in the study after obtaining the consent of their parents. This was a joint study by the newborn clinic, ophthalmology clinic and biochemistry clinic, and the principles of the Declaration of Helsinki were complied.

A Coqun® drop was administered twice a day to the eye's lower fornix between weeks 31 and 34 when ROP development was first observed in the infant's retinas. Fundus examinations were performed weekly following the initiation of Coqun®2x1 treatment. Thirty minutes before the examination, three drops of 0.5% tropicamide (tropamid, Bilim İlaç, Turkey) and 1% phenylephrine (Mydfrin, Alcon, USA) topical ophthalmic solutions were administered to the infants. After appropriate pupillary dilation, topical anaesthesia was performed using proparacaine hydrochloride (Alcaine®, Alcon, USA). Eyelids were opened using eye speculum, and all central and peripheral retinal areas were scanned by indirect ophthalmoscope and +28 dioptric lens vascular indentation. All images were recorded by Archimedes VGA imaging system (Pronova, Ankara, Turkey), and all examinations and treatments were performed by the same ophthalmologist.

Localisation (zone), amount of involvement (clock dial) and vascular examination findings (plus-preplus) were noted based on the severity of retinopathy and retinopathy between avascular areas according to the International ROP Classification criteria (ICROP): Stage 1 if there is a demarcation line, stage 2 if there is elevation or a 'ridge', stage 3 if there is extraretinal fibrovascular proliferation, stage 4 if there is extrafoveal/fovea-containing partial retinal detachment and stage 5 if there is total retinal detachment [17]. These parameters were used to determine the infants' ROP stages. Aggressive posterior ROP (APROP, type II or rush-type ROP with a more posterior location, rapid progression instead of the conventional stage 1–5 progression and poor prognosis despite early treatment) diagnosis was made in the presence of these findings [18].

Nutritional characteristics of infants were examined in three groups: those being fed breast milk, those fed a mix of breast milk and infant formula and those fed infant formulae only. Treatment of the infants was based on the Early Treatment for ROP (ETROP) criteria and BEAT ROP study data [19, 20].

The dose of intravitreal bevacizumab (IVB) (Altuzan® 100 mg/dl, Roche, Switzerland) was 0.16–0.32 mg (very low–low) for all infants [21]. It was administered under local anaesthesia and sterile operating room conditions with 0.5% propargain hydrochloride drop (Alcaine®, Alcon, USA) using a 32-G needle through the limbus's periphery (0.5–1 mm) under topical anaesthesia. Laser photocoagulation (LPC) was performed in a sterile operating room, under sedation or general anaesthesia, using a laser device (Iridex; Oculight SL, Mountainview, CA, USA) on avascular regions for patients in group 2 and above who required this procedure. Coqun® drops were administered twice a day to ROP infants with or without anti-VEGF treatment with the same regimen.

Blood samples were obtained from all infants before and two weeks after Coqun® treatment. Centrifuged blood samples were stored at −80°C until analysis. The albumin level was studied using an Olympus AU 2700 autoanalyser. FRAP was measured using an adapted ELISA method to determine the total antioxidant level [22]. Serum IMA levels were measured using a colorimetric assay method previously described by Bar-Or *et al*. [13]. Thiol-disulphide homeostasis levels were measured using the automated direct measurement method used by Erel *et al*. [14].

The Statistical Package for Social Sciences version 21.0 software for Windows (IBM, Chicago, IL, USA) was used for data analysis. A paired-sample t-test was used for comparisons. Descriptive statistics were expressed as frequency and percentage. Normally distributed quantitative data were expressed as mean ± standard deviation. Non-normally distributed quantitative data were expressed as mean (range). $P < 0.05$ was considered statistically significant.

3. DISCUSSION

Despite a limited number of cases in this study, the significant decrease in serum FRAP levels, which is an indicator of total protective antioxidant level, may lead to increased oxidative stress and progression of this disease.

A decrease in serum FRAP levels indicates that these infants are under severe oxidative stress. The FRAP method, which is an iron (III) ion-reducing antioxidant power method, is suitable for determining hydrophilic and lipophilic antioxidants and measuring the sum of all antioxidants in the environment, excluding thiols [3].

CoQ10s have a high molecular weight and a lipophilic structure that is almost too strong to melt in an aqueous environment. Although there are studies suggesting that CoQ10 has poor bioavailability because it is not water-soluble, there are other studies that show its benefits many organs in the body [11]. Its strong lipophilic structure limits its therapeutic value [23 - 25].

Although there is no study investigating FRAP levels in the blood following topical CoQ10 treatment in newborns and preterm infants, one study reported serum CoQ10 levels in newborns and preterm infants [22]. In this study, CoQ10 initially showed a very low level but increased in vivo in the serum with the presence of vitamin E, which is a potent antioxidant. In newborns exposed to oxidative stress without any external treatment, this increase was caused only by the nutrition and CoQ10 levels produced by the body itself. This increase occurred much slower in preterm infants with a disease, which was attributed to the delay in enteric feeding (*i.e*., a delay in intestinal maturation) [6].

CoQ10 inhibits haemolysis of erythrocytes by hydrogen peroxide in preterm infants by preventing membrane phospholipid peroxidation and thereby free radical formation. It also strengthens defence against oxidative aggression in preterm infants [2].

Oxidative stress plays a critical role in retinal injury and the prognosis of ROP, diabetic retinopathy and ischaemic oxidative retinopathies, such as age-related macular degeneration (AMD); therefore, CoQ10 has an important role in all of these oxidative retinopathies [3]. In their recently published study, Beharry *et al*. demonstrated that CoQ10 and n-3 PUFA supplementation in IH elicited protective effects against ROP [11]. CoQ10 reduced VEGF expression, maintained astrocytic integrity, reduced neovascularisation and normalised retinal layers. In addition, n-3 PUFAs reduced VEGF expression, promoted IGF-I secretion, reduced neovascularisation and normalised retinal layers [11].

Inhibition of ROS production by intravitreal luteolin injection, another potential antioxidant, has reduced VEGF gene transcription [26]. Increasing antioxidant gene expression regulated by Nrf2 *via* intraperitoneal ebselen (a mimic of glutathione peroxidase) injection protected retinal Müller cells and vessels from oxidative stress-induced injury, and led to reduced retinal VEGF expression due to oxidative stress [27].

ROP is an ischaemic retinopathy. Combining CoQ10 and opoloxyethanyl-alpha-tocopheryl sebacate α- water-soluble α-tocopherol (vitamin E) α- with a non-covalent bond at a ratio of 2:1 produces a strong hydrophilic substance. Clinical and laboratory studies have proven that it passes through the corneal epithelium into the eye [16].

In premature infants, IH can result in the disruption of the antioxidant system, leading to disruption of dopamine secretion and thereby neurological damages and increased retinal neovascularisation. The antioxidant effect caused by CoQ10

Table 1. Serum antioxidant levels in infants before and 2 weeks after the initiation of Coqun® treatment.

–	Before Coqun®	After Coqun®	p
FRAP (µmol/L)	0.91 ± 0.17	0.79 ± 0.21	0.006
Albumin (g/dL)	3.6 ± 1.42	4.2 ± 1.17	0.123
IMA (ABSU)	0.85 ± 0.29	0.73 ± 0.36	0.096
Native thiol (µmol/L)	248 ± 38.9	262 ± 42.6	0.164
Total thiol (µmol/L)	284 ± 39.2	291 ± 43.6	0.344
Disulphide (µmol/L)	17.8 ± 6.2	13.5 ± 6.8	0.165
Index 1 (%)	7.45 ± 3.31	5.54 ± 2.49	0.140
Index 2 (%)	6.35 ± 2.4	4.91 ± 1.85	0.124
Index 3 (%)	87.2 ± 4.81	90.1 ± 3.7	0.124

FRAP: Ferric-reducing ability of plasma; IMA: Ischemic modified albumin; ABSU: Absorbance unit.

treatment prevents this neurological damage, protects both inner retina and visual cortex and reduces neurodegenerative damage [11].

Sato *et al*. showed that CoQ10 levels were low at birth, and that CoQ10 plasma levels rapidly increased in the neonatal period when administered in combination with another strong antioxidant, such as vitamin E. This increase was slower in preterm infants with much lower birth weight and gestational age, which was attributed to the late onset of enteric feeding [6].

No significant difference was found between the pre-Coqun and post-Coqun groups in terms of total thiol, native thiol, disulphide, native/total thiol ratio and disulphide/total thiol levels. Based on these results, Coqun® treatment (one drop twice a day) did not alter the systemic serum thiol-disulphide homeostasis. The concentration of thiol-based antioxidants in plasma is lower than in cells: they are formed by serum albumin-SH (~80%) and low molecular weight thiols (*i.e*., cysteine, cysteinylglycine, gamma glutamylcysteine, glutathione and homocysteine). In addition to oxidative stress, many factors affect the plasma thiol/disulphide homeostasis, which may have affected the outcomes of ROP patients [28].

Most recent animal studies have shown that growth is significantly impaired in cases where IH is induced, whereas oral CoQ10 treatment can decrease ROS accumulation by stimulating antioxidant activity and n-3 PUFA treatment can decrease VEGF induced by IH, and therefore, may have beneficial effects on growth and growth-supporting factors [6].

ROP is ischaemic retinopathy. Studies show that CoQ10 can achieve T-fam protein expression in ischaemic retina to provide neuroprotection against mitochondrial changes mediated by oxidative stress in ischaemic retinal damage [14]. Increased reactive oxygen species can activate the inflammatory response by triggering cytokines (inflammatory cytokines, such as IL-1β, TNF-α and IL-6), resulting in oedema development in endothelial cells, decreased blood flow and increased ischaemia, thereby accelerating neovascularisation [25].

Conflicting reports have been published regarding vitamin E supplementation and its use in ROP treatment or prevention. Two meta-analyses have reported beneficial effects, while another study has reported no effect [29 - 31].

Some limitations of this study include the limited number of patients and non-generalisability of the results, as this was a single-centre study.

Although the serum antioxidant levels decreased following the use of topical Coqun® 2 x 1 treatment, the clinical course of ROP in infants was much more stable. Even if serum antioxidant levels do not rise, these levels increase in the vitreous fluid, which may be associated with stabilisation in ROP level. Because the antioxidant levels in the infants' vitreous fluid cannot be checked, investigating these parameters in multi-centre clinical studies or animal models may provide more definitive results. However, the results of this study suggest that administration of CoQ10 not only topically but also orally may be beneficial in the clinical prognosis of infants with this condition.

CONCLUSION

Twenty-eight premature infants with a mean gestational age of 27.6 ± 1.99 (24–32) weeks, a mean birth weight of 1012 ± 318 (520–1750) g and a mean duration of care in the incubator of 64 ± 28 (15–120) days were included in the study.

Three of the infants were breastfed, seven were fed infant formula and 18 were fed both. All infants were in ROP stages 2–3; none had stage 4A and above ROP. Two infants had necrotising enterocolitis and six had APROP findings. One infant was O_2 dependent.

Coqun® treatment (one drop twice a day) was initiated in the newborn unit at gestational weeks 31 and 34, when the initial ROP stages were detected in the infants. Blood antioxidant levels were measured when treatment was initiated and two weeks after initiation. Table **1** shows the serum antioxidant levels of the infants before and two weeks after the Coqun® treatment.

Fundus examinations were performed every week following the initiation of Coqun® treatment. None of the infants progressed in their ROP stage. A total of eight infants (six with APROP and two with stage 3 ROP) had to receive a low dose of IVB injection (0.312 mg) at a mean age of 36.7 weeks.

After two weeks of treatment, the ROP stage regressed in two infants with stage 2 plus (+) ROP. No progression was seen in the other infants; however, eight infants with APROP and stage 3 plus (+) ROP had to receive a low dose of IVB. No infant was treated with LPC.

LIST OF ABBREVIATIONS

Coqun®	=	Coenzyme Q10 and vitamin E
ROP	=	Retinopathy Of Prematurity
FRAP	=	Ferric-Reducing Ability of Plasma
IMA	=	Ishaemia Modified Albumin
O_2	=	Oxygen
IH	=	Intermittent Hypoxia
ROS	=	Reactive O_2 Species
CoQ10	=	Coenzyme Q10
APROP	=	Aggressive Posterior ROP
IVB	=	Intravitreal Bevacizumab
LPC	=	Laser Photocoagulation
AMD	=	Age-Related Macular Degeneration
VEGF	=	Vaso-Endothelial Growth Factor

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The study has been approved by the Ethics Committee of HSU Bursa Yuksek Ihtisas Training and Research Hospital.

HUMAN AND ANIMAL RIGHTS

No Animals were used in this research. All human research procedures followed were in accordance with the ethical

standards of the committee responsible for human experimentation (institutional and national), and with the Helsinki Declaration of 1975, as revised in 2013.

CONSENT OF PUBLICATION

An informed written consent was taken from all the patients when they were enrolled.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

FUNDING

None.

ACKNOWLEDGEMENTS

Muberra Akdogan: Performed study, designed study, collected data and wrote paper.

Yasemin Ustundag: Designed study, analysed data and wrote paper.

Arzu Akdag: Collected data.

Ozcan Erel, Yasemin Ustundag, Salim Neselioglu: Study sample

English editing was performed by Enago and Scribendi editing service.

REFERENCES

[1] Shah PK, Prabhu V, Karandikar SS, Ranjan R, Narendran V, Kalpana N. Retinopathy of prematurity: Past, present and future. World J Clin Pediatr 2016; 5(1): 35-46. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4737691].
[http://dx.doi.org/10.5409/wjcp.v5.i1.35] [PMID: 26862500]

[2] Hara K, Yamashita S, Fujisawa A, Ishiwa S, Ogawa T, Yamamoto Y. Oxidative stress in newborn infants with and without asphyxia as measured by plasma antioxidants and free fatty acids. Biochem Biophys Res Commun 1999; 257(1): 244-8. [https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6160725].
[http://dx.doi.org/10.1006/bbrc.1999.0436] [PMID: 10092541]

[3] Rivera JC, Dabouz R, Noueihed B, Omri S, Tahiri H, Chemtob S. Ischemic retinopathies: Oxidative stress and inflammation. Oxid Med Cell Longev 2017; 2017: 3940241.
[http://dx.doi.org/10.1155/2017/3940241] [PMID: 29410732]

[4] Akdogan M, Demirag DA, Varal IG, Cevik SG, Ustundag Y. Haemogram parameters in the development of retinopathy of prematurity. Open J Ophthalmol 2018; 8: 75-83.
[http://dx.doi.org/10.4236/ojoph.2018.82011]

[5] Blokhina O, Virolainen E, Fagerstedt KV. Antioxidants, oxidative damage and oxygen deprivation stress: A review. Ann Bot 2003; 91(Spec No): 179-94.
[http://dx.doi.org/10.1093/aob/mcf118] [PMID: 12509339]

[6] Sato S, Tsukahara H, Ohshima Y, *et al.* Changes of plasma coenzyme Q10 levels in early infancy. Redox Rep 2004; 9(5): 289-90.
[http://dx.doi.org/10.1179/135100004225006768] [PMID: 15606982]

[7] Rivera JC, Holm M, Austeng D, *et al.* Retinopathy of prematurity: Inflammation, choroidal degeneration, and novel promising therapeutic strategies. J Neuroinflammation 2017; 14(1): 165.
[http://dx.doi.org/10.1186/s12974-017-0943-1] [PMID: 28830469]

[8] Manke A, Wang L, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. BioMed Res Int 2013; 2013: 942916.
[http://dx.doi.org/10.1155/2013/942916] [PMID: 24027766]

[9] Hartnett ME. Pathophysiology and mechanisms of severe retinopathy of prematurity. Ophthalmology 2015; 122(1): 200-10.https://www.ncbi.nlm.nih.gov/pubmed/25444347
[http://dx.doi.org/10.1016/j.ophtha.2014.07.050] [PMID: 25444347]

[10] Al-Shabrawey M, Bartoli M, El-Remessy AB, *et al.* Inhibition of NAD(P)H oxidase activity blocks vascular endothelial growth factor overexpression and neovascularization during ischemic retinopathy. Am J Pathol 2005; 167(2): 599-607.
[http://dx.doi.org/10.1016/S0002-9440(10)63001-5] [PMID: 16049343]

[11] Beharry KD, Cai CL, Henry MM, Chowdhury S, Valencia GB, Aranda JV. Co-Enzyme Q10 and n-3 polyunsaturated fatty acid supplementation reverse intermittent hypoxi-induced growth restriction and improved antioxidant profiles in neonatal rats. Antioxidants 2017; 6(4): 103.
[http://dx.doi.org/10.3390/antiox6040103] [PMID: 29258174]

[12] Zmitek J, Smidovnik A, Fir M, *et al.* Relative bioavailability of two forms of a novel water-soluble coenzyme Q10. Ann Nutr Metab 2008; 52(4): 281-7.
[http://dx.doi.org/10.1159/000129661] [PMID: 18645245]

[13] Bar-Or D, Lau E, Winkler JV. A novel assay for cobalt-albumin binding and its potential as a marker for myocardial ischemia-a preliminary report. J Emerg Med 2000; 19(4): 311-5.
[http://dx.doi.org/10.1016/S0736-4679(00)00255-9] [PMID: 11074321]

[14] Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004; 37(4): 277-85.
[http://dx.doi.org/10.1016/j.clinbiochem.2003.11.015] [PMID: 15003729]

[15] Unal S, Ulubas Isik D, Bas AY, *et al.* Evaluation of dynamic thiol-disulfide homeostasis in very low-birth-weighted preterms. J Matern Fetal Neonatal Med 2017; 13: 1-6.
[PMID: 29092682]

[16] Fato R, Bergamini C, Leoni S, *et al.* Coenzyme Q10 vitreous levels after administration of coenzyme Q10 eyedrops in patients undergoing vitrectomy. Acta Ophthalmol 2010; 88(4): e150-1.
[http://dx.doi.org/10.1111/j.1755-3768.2009.01632.x] [PMID: 19799594]

[17] International Committee for the classification of Retinopathy of Prematurity: The international classification of Retinopathy of Prematırity revisited. Arch Opthalmology 2005; 123: 991-9.
[http://dx.doi.org/10.1001/archopht.123.7.991]

[18] Zhou J, Liu Z, Ying HY, Liu T. Aggressive posterior retinopathy of prematurity in a premature male infant. Case Rep Ophthalmol 2017; 8(2): 396-400.
[http://dx.doi.org/10.1159/000478694] [PMID: 28924435]

[19] Good WV. Final results of the early treatment for Retinopathy of Prematurity (ETROP) randomized trial. Trans Am Ophthalmol Soc 2004; 102: 233-48.
[PMID: 15747762]

[20] Kennedy KA, Mintz-Hittner HA. Medical and developmental outcomes of bevacizumab versus laser for retinopathy of prematurity. J AAPOS 2018; 22(1): 61-65.e1.
[http://dx.doi.org/10.1016/j.jaapos.2017.10.006] [PMID: 29223789]

[21] Wallace DK, Kraker RT, Freedman SF. Assesment of lower dose of intravitreous bevacizumab for retinopathy of prematurity. JAMA Opthal 2017; 135: 656-65.

[22] Ustundag Y, Huysal K, Kahvecioglu S, *et al.* Establishing reference values and evaluation of an in-house Ferric Reducing Antioxidant Power (FRAP) colorimetric assay in microplates. Eur Respir J 2016; 2: 126-31. [https://www.researchgate.net/.../304352516].

[23] Constantinescu R, McDermott MP, Dicenzo R, *et al.* A randomized study of the bioavailability of different formulations of coenzyme Q(10) (ubiquinone). J Clin Pharmacol 2007; 47(12): 1580-6.
[http://dx.doi.org/10.1177/0091270007307571] [PMID: 17925590]

[24] Bhagavan HN, Chopra RK. Plasma coenzyme Q10 response to oral ingestion of coenzyme Q10 formulations. Mitochondrion 2007; 7(Suppl.): S78-88.
[http://dx.doi.org/10.1016/j.mito.2007.03.003] [PMID: 17482886]

[25] Sikorska M, Borowy-Borowski H, Zurakowski B, Walker PR. Derivatised α-tocopherol as a CoQ10 carrier in a novel water-soluble formulation. Biofactors 2003; 18(1-4): 173-83.
[http://dx.doi.org/10.1002/biof.5520180220] [PMID: 14695933]

[26] Park SW, Cho CS, Jun HO, *et al.* Anti-angiogenic effect of luteolin on retinal neovascularization *via* blockade of reactive oxygen species production. Invest Ophthalmol Vis Sci 2012; 53(12): 7718-26.
[http://dx.doi.org/10.1167/iovs.11-8790] [PMID: 23099493]

[27] Tan SM, Deliyanti D, Figgett WA, Talia DM, de Haan JB, Wilkinson-Berka JL. Ebselen by modulating oxidative stress improves hypoxia-induced macroglial Müller cell and vascular injury in the retina. Exp

Eye Res 2015; 136: 1-8.
[http://dx.doi.org/10.1016/j.exer.2015.04.015] [PMID: 25912997]

[28] Turell L, Radi R, Alvarez B. The thiol pool in human plasma: The central contribution of albumin to redox processes. Free Radic Biol Med 2013; 65: 244-53.
[http://dx.doi.org/10.1016/j.freeradbiomed.2013.05.050] [PMID: 23747983]

[29] Raju TN, Langenberg P, Bhutani V, Quinn GE. Vitamin E prophylaxis to reduce retinopathy of prematurity: A reappraisal of published trials. J Pediatr 1997; 131(6): 844-50.
[http://dx.doi.org/10.1016/S0022-3476(97)70031-3] [PMID: 9427888]

[30] Brion LP, Bell EF, Raghuveer TS. Vitamin E supplementation for prevention of morbidity and mortality in preterm infants. Cochrane Database Syst Rev 2003; CD003665.

[31] Law MR, Wijewardene K, Wald NJ. Is routine vitamin E administration justified in very low-birthweight infants? Dev Med Child Neurol 1990; 32(5): 442-50.
[http://dx.doi.org/10.1111/j.1469-8749.1990.tb16963.x] [PMID: 2191890]